REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow. Claims 55 and 56 are requested to be cancelled. Claims 1, 16-19 and 30 are currently being amended. This amendment adds, changes and/or deletes claims in this application. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, with an appropriate defined status identifier. The Official Action incorrectly indicates the list of pending claims on both the cover sheet and on page 2. Following this amendment, claims 1, 12-20, 30, and 51-54 are pending.

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would further advance the prosecution of the present application.

Claims 1, 12-20, 30, 51, 52, 55 and 56 are rejected under the second paragraph of Section 112 as being indefinite. "The target site" in claims 1 and 30 has been amended to recite "the targeted tissue," for which antecedent basis is found in element (A) of the claims.

Claims 16-19 are rejected under the second paragraph of Section 112 as being indefinite. Claims 16, 18 and 19 have been amended to recite that first or second targetable conjugate comprises a peptide, hapten or chelator or metal-chelate complex, respectively, to which said at least one other arm of said bi-specific antibody binds. Claim 17 has been amended to recite that the second targetable conjugate comprises a carrier portion comprising a carbohydrate.

Claim 20 is rejected under the first and second paragraphs of Section 112 and under Section 101, based on the its recitation of a detectable radionuclide in a method of treating. However, the specification makes it clear that a conjugate may comprise, for example, both a diagnostic radionuclide and a therapeutic radionuclide ("More than one type of chelator may be conjugated to a carrier to bind multiple metal ions, *e.g.*, non-radioactive ions, diagnostic radionuclides and/or therapeutic radionuclides. One example is a bis-111In-DTPA conjugate that also bears a DOTA-90Y chelate." – Specification at page 23, lines 25-29) The combination enables both imaging and therapy following administration of a single agent. It allows a clinician to track where an administered therapeutic is localizing in the body, a readily recognizable utility easily understood and implemented by a skilled artisan. Accordingly, the alleged bases for these rejections are not understood.

Claims 1, 12-16, 18-20, 30 and 51 are rejected under Section 103(a) based on Barbet *et al.* (EP 0,263,046) or Goodwin *et al.* (US 4,863,713), either in view of Bagshawe *et al.* (U.S. 5,683,694). Claims 16 and 17 are rejection under the same combination, taken in further view of Wagner *et al.* (U.S. 5,503,987). The examiner urges that Barbet or Goodwin teach the method of instant claim 1, except for the fact that neither contemplates the use of prodrugs. The examiner argues that based on Bagshawe's teaching that "an enzyme that activates a prodrug is known to fall within the armamentarium of therapeutic agents that can be targeted by bispecific antibodies, it would have been obvious to provide the enzyme of Bagshawe *et al.* conjugated o a hapten/epitope recognized by the antibody in the second arm of the bispecific antibodies of Barbet *et al.* or Goodwin *et al.*" In this regard he notes that "the examiner considers the lack of teaching of prodrug activating enzymes as being among the therapeutic agents listed by Barbet *et al.* or Goodwin *et al.* arise from the fact that prodrug activating enzymes were not well established as therapeutic agents at the time that Barbet *et al.* and Goodwin *et al.* filed, rather than from any consideration that such enzymes would not be compatible with their taught targeting methods."

Barbet and Goodwin both were filed in 1986. However, both continued to work in this field, and both authored several later articles, circa 1996-1999, in which the use of pretargeting was further explored. None of these later articles expanded the use of pretargeting to encompass the use of enzymes and prodrugs.[1] Indeed, the use of enzyme/prodrug embodiments is conspicuously absent from a review of "Pretargeting: general principles" presented by Goodwin and Maeres in October 1996 (Nuclear Medicine Service, Veterans Affairs Palo Alto Health Care System, California). Similarly, articles on which Barbet is a co-author that appeared circa 1997/1998, the time frame of applicants' filing date, all relate to bivalent hapten-bearing peptide designed for iodine-121 pretargeted radioimmunotherapy. Not until October 2001, ***three years after applicant's priority date***, do either Barbet or Goodwin mention anything about enzymes and prodrugs in the context of a pretargeting regimen. At that time, Goodwin and Maeres co-authored an article entitled "Advances in pretargeting biotechnology" which mentions antibody-directed enzyme prodrugs in a pretargeting context.

Accordingly, it is submitted that the failure of both Barbet *et al.* or Goodwin *et al.* to envision prodrug activating enzymes as being among the therapeutic agents useful in pretargeting is due not only due to the fact that prodrug activating enzymes were not well established as therapeutic agents at

[1] Copies of various articles by Barbet, Goodwin, and Bagshawe that are referenced herein are appended.

the time of their applications, but also to the fact that such a substitution simply ***would not have been obvious***. Bagshawe *et al.* were publishing articles on ADEPT circa 1990, well before the time frame of the later articles published by Barbet and Goodwin in 1997/1998. Yet it was not until after applicants' invention that Goodwin *et al.* put the pieces together in his 2001 article. It appears that the hindsight in this case truly is 20:20. Based on this, the examiner is urged to reconsider his conclusion that "the lack of teaching of prodrug activating enzymes as being among the therapeutic agents listed by Barbet *et al.* or Goodwin *et al.* arise from the fact that prodrug activating enzymes were not well established as therapeutic agents at the time that Barbet *et al.* and Goodwin *et al.* filed, rather than from any consideration that such enzymes would not be compatible with their taught targeting methods."

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date October 20, 2003

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J Nucl Med. 1998 Nov;39(11):1937-43.

Delivery of therapeutic doses of radioiodine using bispecific antibody-targeted bivalent haptens.

Gautherot E, Le Doussal JM, Bouhou J, Manetti C, Martin M, Rouvier E, Barbet J.

Immunotech SA, Marseille, France.

Two-step pretargeting strategies have been designed to deliver radioisotopes to tumors more selectively than directly labeled antibodies or fragments. In this article, we compare quantitatively the potential of these strategies for the radioimmunotherapy of solid tumors. METHODS: Direct targeting was performed using iodine-labeled IgG and F(ab')2. As two-step strategies, we used the sequential injection of anti-CEA x anti-DTPA-In bispecific F(ab')2 (BsF(ab')2) and monovalent and bivalent DTPA derivatives labeled with iodine. The biodistribution of iodine in nude mice grafted with the LS174T human colorectal carcinoma was monitored in time and used for calculating radiation doses. RESULTS: In agreement with earlier studies, the IgG was more effective for delivering a radiation dose to the tumor than the F(ab')2 (7.8 versus 0.76 Gy/MBq, respectively) and both were moderately selective with respect to normal tissues (tumor:blood of 2.9 and 1.7, respectively). At their MTD, they should deliver 86 and 34 Gy, respectively, to the tumor. Using a nM-affinity DTPA-In bivalent hapten, the two-step protocol was optimized by varying the dosage of the BsF(ab')2, the stoichiometry of the reagents and the pretargeting time. The saturation of the tumor was obtained by injecting 5 nmol (500 microg) of BsF(ab')2. The pretargeted BsF(ab')2 was saturated by the injection of 0.5 mol of bivalent hapten per mole of antibody. With a 48-hr pretargeting time, the selectivity of the irradiation of the tumor was optimized (tumor:blood of 7.8) but only at the price of a lower efficiency (0.35 versus 0.86 Gy/MBq, 48-hr and 20-hr pretargeting time, respectively). Attempts to increase selectivity by using a microM-affinity DTPA-Y bivalent hapten or by chasing excess circulating radiolabeled hapten with an excess of unlabeled hapten also reduced tumor exposure. The use of a monovalent hapten resulted in both lower efficiency and selectivity. However, the two-step pretargeting of high-affinity bivalent hapten (Affinity Enhancement System, AES) should deliver 30-60 Gy to the tumor with less than 9 Gy to the blood in tumor-bearing mice. CONCLUSION: Radioimmunotherapy with AES is predicted to be as efficient and with lower hematological toxicity than direct targeting.

PMID: 9829586 [PubMed - indexed for MEDLINE]

Bioconjug Chem. 1997 Jul-Aug;8(4):526-33.

ACS PUBLICATIONS

Bivalent hapten-bearing peptides designed for iodine-131 pretargeted radioimmunotherapy.

Janevik-Ivanovska E, Gautherot E, Hillairet de Boisferon M, Cohen M, Milhaud G, Tartar A, Rostene W, Barbet J, Gruaz-Guyon A.

INSERM U.339, Paris, France.

Pretargeting with bispecific antibodies has been used successfully for tumor detection and is now considered for radioimmunotherapy. The advantages of bivalent haptens have been demonstrated in this context. A series of bivalent molecules allowing efficient labeling with radioactive iodine has been designed for use with this new technology. They were based on the histamine-hemisuccinate hapten and prepared by solid phase peptide synthesis. Simultaneous binding of two antibody molecules to one bivalent hapten was possible with low steric hindrance when the two hapten groups were attached to the lateral chains of lysine residues separated by a single amino acid. Bispecific antibodies to the hapten and to carcinoembryonic antigen were shown to mediate specific binding of the haptens to tumor cells in vitro. These experiments demonstrated that the bivalent hapten AG3.0, with a lysyl-D-tyrosyl-lysine connecting chain, possessed the best binding properties. This peptide was used to target iodine-125 to human colon cancer xenografts in nude mice. High tumor uptake and tumor to normal tissue ratios were observed. This peptide thus appears as a good candidate for further development. Asymmetric bivalent haptens, with one histamine-hemisuccinate and one diethylenetriaminepentaacetic acid group, have also been prepared and shown to be capable of binding simultaneously two specific antibody molecules. These peptides should be useful to target radioiodine to cells characterized by the expression of two different antigenic markers.

PMID: 9258451 [PubMed - indexed for MEDLINE]

Cancer. 1997 Dec 15;80(12 Suppl):2618-23.

Therapy for colon carcinoma xenografts with bispecific antibody-targeted, iodine-131-labeled bivalent hapten.

Gautherot E, Bouhou J, Le Doussal JM, Manetti C, Martin M, Rouvier E, Barbet J.

IMMUNOTECH SA, Marseille, France.

BACKGROUND: One of the main limitations of radioimmunotherapy (RIT) is the secondary toxicity related to the poor therapeutic indices achieved with labeled whole immunoglobulin (Ig)G or F(ab')2 fragments. To overcome this problem, we have developed a two-step targeting method, which we refer to as the Affinity Enhancement System (AES), using a radiolabeled bivalent hapten and a bispecific antibody recognizing the hapten and a target cell antigen. This method has been applied successfully to immunoscintigraphy in carcinoembryonic antigen (CEA)-expressing carcinoma patients and increased tumor to normal tissue uptake ratios have been achieved. The aim of the current study was to evaluate the application of AES to RIT of CEA-expressing solid tumors in an animal model. METHODS: Nude mice grafted with LS174T human colorectal carcinoma were treated either with 111 megabecquerels (MBq) of iodine-131 labeled bivalent diethylenetriamine pentaacetic acid (DTPA) hapten 20 hours after pretargeting by anti-CEA x anti-DTPA-indium bispecific antibody or 12 MBq of iodine-131 labeled anti-CEA IgG. RESULTS: Treatment with the IgG induced only a growth delay of 53 +/- 5 days but all tumors progressed. Treatment with the AES was highly efficient because tumor growth inhibition was achieved over 150 days. Hematologic and overall toxicity of both treatments were equivalent. CONCLUSIONS: The long term tumor regression consecutive to AES RIT represents a very significant improvement over the use of directly labeled IgG. Toxicity consecutive to AES or IgG RIT were similar despite an administered activity nearly ten times higher with the AES. However, given the efficacy of the AES treatment, a lower dose may afford lower toxicity and significant antitumor effect.

PMID: 9406716 [PubMed - indexed for MEDLINE]

Pretargeting: general principles; October 10-12, 1996.

Goodwin DA, Meares CF.

Nuclear Medicine Service, Veterans Affairs Palo Alto Health Care System, California 94304, USA.

BACKGROUND: Human imaging studies show that maximum human tumor concentrations of monoclonal antibody (MoAb) are achieved in 1 day, but several days are required for background reduction and sensitive radioimmunoscintigraphy of tumors. With therapeutic radionuclides such as yttrium-90 (90Y), this long biologic half-life imposes a high radiation burden on sensitive normal tissues from the large amount of unlocalized radioactivity. Normal tissue toxicity, especially to the bone marrow, has been the major limiting factor in the application of radioimmunotherapy to solid tumors. Pretargeting techniques provide an alternative method with which to obtain high selective tumor uptake of 90Y with simultaneous minimization of nontarget tissue background. METHODS: Current MoAb techniques employ either the biotin/avidin or the hapten/antibody system. DNA/DNA and prodrug/enzyme systems also have been used and many other ligand/receptor systems are possible. All the methods depend on a long circulating conjugate to obtain high target uptake with a diffusible, rapidly excreted effector molecule. RESULTS: Fast in, slow out tumor kinetics, ideal for therapy, have been achieved. In one mouse tumor system the biologic half-life of a bivalent 88Y JANUS tetraazacyclodidecanetetraacetic acid hapten, measured over 5 days, was approximately 24 hours. The therapeutic ratio, obtained from the integrated tumor and blood concentrations over 5 days, was approximately 20/1 compared with 2-3/1 with directly labeled MoAb. The total injected dose remaining in the mouse at 24 hours was 5.5%, 23% of which was in the tumor. CONCLUSIONS: These result suggest that it may be possible to deliver tumoricidal radiation doses with 90Y using pretargeting techniques without severe normal bone marrow irradiation.

PMID: 9406724 [PubMed - indexed for MEDLINE]

J Nucl Med. 1998 Oct;39(10):1813-8.

Biological properties of biotin-chelate conjugates for pretargeted diagnosis and therapy with the avidin/biotin system.

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Three-step pretargeting increases target-to-background ratios in radioimmunodetection and can potentially decrease harmful radiation to normal tissues in radioimmunotherapy. We studied four biotin-chelate conjugates (BCCs) for use in the avidin/biotin pretargeting system. METHODS: Pharmacokinetics and biodistribution were studied in normal BALB/c (IAk-negative), normal C3H (IAk-positive) and LS174T tumor-bearing BALB/c severe combined immunodeficient mice. Streptavidin alone and antibody-streptavidin conjugates [monoclonal antibody (MAb) 10-3.6 anti-IAk IgG2a] were used. Indium-111- or 88Y-BCCs were given alone intravenously; they were mixed with streptavidin or MAb-streptavidin conjugate and given intravenously; or streptavidin and MAb-streptavidin conjugate were pretargeted, and 2-3, 5 and 21 hr later, BCCs were injected intravenously. Samples were taken 2-3 hr after intravenous injection of labeled BCCs. RESULTS: Three of the four BCCs were rapidly excreted by the kidneys, with <2.5%/g in any organ or tumor at 2-3 hr. Gut excretion eliminated biotinyl-(S)-1-p-aminobenzylethylenediaminetetraacetic acid (EDTA) for use in pretargeting. Ninety percent of BCCs were bound to circulating pretargeted streptavidin at 1-6 hr, and approximately 15% were bound to pretargeted streptavidin at 24 hr. Kidney uptakes were: preformed streptavidin-BCC given intravenously, approximately 80%/g (24 hr); streptavidin pretargeted for 2-3 hr, approximately 60%/g; and streptavidin pretargeted for 5-21 hr, approximately 10%-20%/g. Kidney uptake was dose-dependent: 0.2, 0.67 and 1.0 nmol of streptavidin pretargeted for 21 hr showed increasing concentrations (24 hr). Uptake of monoclonal anti-IAk-streptavidin-BCC complex into spleen (70% +/- 10%/g; $p < 0.05$) and lymph nodes (10% +/- 3.5%/g; $p < 0.01$) was higher in IAk-positive C3H mice than it was in IAk-negative control BALB/c mice, and it was much higher than that in streptavidin controls. No significant target uptake was seen with anti-IAk MAb-streptavidin pretargeted for 3 or 20 hr. Kidney uptake approximately 20%/g, which was lower than that of streptavidin alone. CONCLUSION: Three biotinyl chelates bind the diagnostic and therapeutic radiometals 111In and 88Y (and, by analogy, 90Y) with the required in vivo stability and physiological properties for pretargeted diagnosis and therapy. Kidney uptake of streptavidin was decreased by conjugation to MAb. Failure of anti IAk MAb-streptavidin conjugate to bind BCC after pretargeting may be due to rapid internalization of MAb-streptavidin-IAk complex by the lymphocyte or to endogenous biotin. Either or both of these would make streptavidin unavailable to subsequent BCCs.

PMID: 9776294 [PubMed - indexed for MEDLINE]

Biotechnol Adv. 2001 Oct;19(6):435-50.

Advances in pretargeting biotechnology.

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Nuclear Medicine Service, Veterans Affairs Palo Alto Health Care System, 94304, Palo Alto, CA, USA

A major focus of current drug research is to improve drug targeting to internal target sites such as to solid tumors or specific organs. The objective of drug targeting, especially for cancer chemotherapy and radioimmunotherapy, is to enhance the effectiveness of the drug by concentrating it at the target site and minimizing its effects in nontarget sites. Although tumor targeting has been obtained with large long-circulating radiolabeled antibody molecules, normal organ activity, especially in the blood kidneys, liver, and bone marrow is a significant problem. Over the last 20 years, studies to improve the therapeutic use of antibodies have included the use of antibody fragments, chase molecules, metabolizable linkers, antibody-directed enzyme prodrugs (ADEPT), local delivery, and pretargeting. Here, we will review the most interesting recent advances in pretargeting biotechnology.

PMID: 14538068 [PubMed - in process]

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) A method of treating diseased tissues in a patient, comprising:

(A) administering to said patient a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;

(B) optionally, administering to said patient a clearing composition, and allowing said composition to clear non-localized antibodies or antibody fragments from circulation;

(C) administering to said patient a first targetable conjugate which comprises a carrier portion and one or more conjugated enzymes, wherein said carrier portion comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment; and

(D) administering to said patient

(1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the ~~target site~~ targeted tissue; or

(2) a drug which is capable of being detoxified in said patient to form an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the ~~target site~~ targeted tissue, or

(3) a prodrug which is activated in said patient through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the ~~target site~~ targeted tissue, or

(4) a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and a prodrug, when said enzyme is capable of converting said prodrug to a drug at the ~~target site~~ targeted tissue.

2-11. (Canceled)

12. (Original) The method of claim 1, wherein said at least one arm that specifically binds a targeted tissue is a monoclonal antibody or a fragment of a monoclonal antibody.

13. (Original) The method of claim 1, wherein said at least one other arm that specifically binds a targetable conjugate is a monoclonal antibody or a fragment of a monoclonal antibody.

14. (Original) The method of claim 1, wherein said at least one arm that specifically binds a targeted tissue is a humanized antibody or a fragment of a humanized antibody.

15. (Original) The method of claim 1, wherein said at least one other arm that specifically binds a targetable conjugate is a humanized antibody or a fragment of a humanized antibody.

16. (Currently Amended) The method of claim 1, wherein said first or second targetable conjugate comprises a peptide to which said at least one other arm of said bi-specific antibody binds.

17. (Currently Amended) The method of claim 1, wherein said ~~first or~~ second targetable conjugate comprises a carrier portion comprising a carbohydrate.

18. (Currently Amended) The method of claim 1, wherein said first or second targetable conjugate comprises one or more haptens to which said at least one other arm of said bi-specific antibody binds.

19. (Currently Amended) The method of claim 1, wherein said first or second targetable conjugate comprises one or more chelators or metal-chelate complexes to which said at least one other arm of said bi-specific antibody binds.

20-29. (Canceled)

30. (Currently amended) A kit useful for treating diseased tissues in a patient comprising:

(A) a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;

(B) a first targetable conjugate which comprises a carrier portion and one or more conjugated enzymes, wherein said carrier portion comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment;

(C) optionally, a clearing composition useful for clearing non-localized antibodies and antibody fragments; and

(D) (1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the ~~target site~~ targeted tissue; or

(2) a drug which is capable of being detoxified in said patient to form an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the ~~target site~~ targeted tissue, or

(3) a prodrug which is activated in said patient through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the ~~target site~~ targeted tissue, or

(4) a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and a prodrug, when said enzyme is capable of converting said prodrug to a drug at the ~~target site~~ targeted tissue.

31-50. (Canceled)

51. (Currently amended) The method of claim 1, wherein (D) comprises administering a prodrug and said enzyme is capable of converting said prodrug to a drug at the ~~target site~~ targeted tissue.

52. (Currently amended) The method of claim 1, wherein (D) comprises administering a prodrug that is activated in said patient through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, and said enzyme is capable of reconverting the detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the ~~target site~~ targeted tissue.

53. (Previously presented) The method of claim 1, further comprising, when said first targetable conjugate additionally comprises a prodrug, administering a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and an enzyme capable of converting said prodrug to a drug or of reconverting a detoxified intermediate of said drug to a toxic form.

54. (Previously presented) The kit of claim 30, further comprising, when said first targetable conjugate additionally comprises a prodrug, a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and an enzyme capable of converting said prodrug to a drug or of reconverting a detoxified intermediate of said drug to a toxic form.

55-56. (Canceled)